TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET, NEW YORK 17, N.Y.

#144

Application For Research Grant

Date: November 13, 1956

1. Name of Investigator: Donald F. McDonald, M.D.
Kenneth P. Knudtson, M.D.
T. Lloyd Fletcher, Ph.D.

2. Title: Experimental Carcinogenicity of Tobacco Smoke Condensate Extractives to Transitional Epithelium of the Urinary Bladder.

3. Institution & Address: University of Washington School of Medicine
Seattle 5, Washington

4. Project or Subject:

The purposes of the project are:

a. To ascertain whether tobacco tar (smoke condensate) extractives are capable of inducing transitional cell tumors of urinary bladder epithelium in mice, rats, and dogs.

b. To identify the substance or substances responsible for a positive result in (a).

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

a. Preparation of smoke condensate extractives (Ecusta Paper Company).

The general scheme of partitioning the smoke condensate into convenient fractions for preliminary testing is as follows:

Tar in suitable solvent

Extract with base - neutralize extract with solvent, dry and evaporate (a)

Residue
Extract with acid - neutralize extract with solvent, dry and evaporate (b)

Residue
Evaporate solvent (c)

(a), (b), (c) will be individually subjected to chromatographic separation, thus, providing $a_{1,2,3}$, $b_{1,2,3}$, $c_{1,2,3}$ and residues.

The objective of preliminary fractionation will be to provide a convenient number of fractions for testing. When testing has been completed, the direction of efforts to identify whatever carcinogen may be found present might be localized to one fraction. Identification of the carcinogen could then be attempted and biologically proved. Because the yield of material in each fraction can be estimated only very roughly, the estimate of amount of

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smoke condensate required cannot be very realistic. Dr. Knudtson is planning to use some of the fractions for a pilot series of intrapulmonic implants to test carcinogenicity of the material to the lung. Pure nicotine will also be tested.

b. Testing of smoke condensate extractives for carcinogenicity.

Because of species differences, we feel that conclusive evaluation of carcinogenicity is best achieved by working with as many species as is practical. For statistically sound results, twenty observations for each substance should be made. We have been working in the field of bladder cancer for seven years. During the past five years, we have been establishing reliable methods for testing carcinogenicity. We are currently testing human urinary solid extractives and residues from patients with urinary bladder tumors, patients with tumors of other organs and normal patients. Dr. Knudtson has helped us in the histological evaluation of some of the material. He has volunteered to help us with histological studies in the present experiment. Because of our experience, we feel we are in an advantageous position to conduct an expeditious, reliable and conclusive evaluation of the carcinogenicity of smoke condensate extractives. The details of methods in the various species follows:

(1) Mice:

Surgical implantation of a pellet of the material incorporated in cholesterol or paraffin can be regarded as a completely satisfactory method of evaluation in this species. Forty to 52 weeks should be a satisfactory induction. Ref.: Bonser, Clayson, Jull and Pyrah, Brit. J. Urol. 26:49-52, 1954.

(2) Rats:

The formation of papillary bladder tumors in rats frequently follows the prolonged stimulus of a urinary calculus. For this reason, Reffo's work cannot be considered reliable. Calculi form about niduses introduced into rat bladders unless they are smooth (e.g., glass). We have found that subcutaneous transplantation of half of the rat bladder provides a bladder pouch, not in contact with the urine, which serves as a very suitable test object for testing of topically effective carcinogens. Tumors can form in as little as six months, however, optimum results are obtained in one year.

(3) Dogs:

By surgical partial obstruction of one ureter, a large dilated ureter, which subsequently can be transplanted to a subcutaneous position, provides room for implantation of up to ten different substances. Colored nylon ligatures permit positive identification of the isolated segments of ureter up to two years later. Bladder mucosa can be stripped, wrapped around a polyethylene splint and free-grafted to the subcutaneous tissues of the chest wall. This provides an additional number of testing pouches. Testing in dogs requires eighteen months.

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6. Budget Plan: For year, 1/1/57 to 12/31/57

Salaries:

Ph.D. Chemist, part-time, 6 mo.	$1,800.00	
Technical Assistant, surgery, histology	3,600.00	$5,400.00

Expendable supplies:

	Initial cost	Food and care		
200 mice	$100.00	$ 50.00		
200 rats	200.00	100.00		
10 dogs	120.00	1,100.00		
			$1,670.00	
Smoke condensate (100 ampoules)			430.00	
Solvents, reagents, glassware			600.00	
Histological supplies			250.00	
Photography			100.00	3,050.00

Equipment: None

SUBTOTAL	$ 8,450.00
Overhead (15%):	1,267.00
	$ 9,717.00

7. Anticipated Duration of Work:

It will probably require six months to prepare the smoke condensate and implant the extractive fractions into the animals. An additional year induction time and six months for preparation, histological evaluation, and summation. During the second year, the expenses will be largely those incident to animal care and feeding, post-mortem and histological preparation and photography. Estimated requirement for the second year is:

Salaries, $3,600.00; animal maintenance, $500.00; supplies, $200.00 -- subtotal, $4,300.00 / 15% overhead of $645.00 -- Totaling $4,945.00.

8. Facilities and Staff Available:

Facilities -- University of Washington Department of Surgery Experimental Animal Surgery.

-- Animal quarters with complete facilities.

-- 14' x 17' chemical laboratory with all facilities, 6' hood, Gramatic balance, #1 International centrifuge, one circular Precision Warburg apparatus with Summerson manometers and vessels, Evelyn colorimeter, Beckman Model B Spectrophotometer.

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--10' x 12' laboratory with facilities for animal handling, post mortem examination, small animal operating, including instruments and sterilizer.

--15' x 12' isotope laboratory with high speed hood, autoscaler and scintillating proportional counters, balance centrifuge, constant temperature bath, hot plates, usual and special glassware.

--12' x 12' office with slide files, binocular research microscope and light.

Staff:

Donald F. McDonald, M.D., Associate Professor of Surgery and Head of the Division of Urology, University of Washington School of Medicine.

K. P. Knudtson, M.D., Assistant Chief, Laboratory Service, U. S. Veterans Administration Hospital, Seattle, Washington.

T. Lloyd Fletcher, Ph.D., Research Associate Professor, Director of the Department of Surgery Chemistry Research Laboratory, University of Washington School of Medicine, Seattle, Washington.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Enclosed are reprints of previous work done in closely related fields, entitled:

"The Role of the Urine in Vesical Neoplasm. I. Experimental Confirmation of the Urogenous Theory of Pathogenesis."

"Clinical Implications of Transplantability of Induced Bladder Tumors to Intact Transitional Epithelium in Dogs."

Because our studies of carcinogenicity of human urine solids have not been completed (we still have 22 dogs to finish), no published material is available.

Signature /s/ Donald F. McDonald
Director of Project

/s/ Henry N. Harkins
Executive Officer, Dept. of Surgery

C. N. Smiley
Dean - School of Medicine

/s/ for: Nelson A. Wilstrom
Comptroller

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